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(54) HUMANIZED IMMUNOGLOBULINS AND THEIR PRODUCTION AND USE

HUMANISIERTE IMMUNGLOBULINE, DEREN HERSTELLUNG UND VERWENDUNG

IMMUNOGLOBULINES HUMANISES, LEURS PRODUCTION ET UTILIZATION

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mains. In general, the production of human immunoglobulins reactive with the human IL-2 receptor, as with many human antigens, has been extremely difficult using typical human monoclonal antibody production techniques. Similarly, past attempts utilizing recombinant DNA technology to produce so-called "humanized" antibodies (see e.g. EPO Publication No. 0239400), provides uncertain results, in part due to unpredictable binding affinities.

Thus, there is a need for improved forms of human-like immunoglobulins, such as those specific for the human IL-2 receptor, that are substantially non-immunogenic in humans, yet easily and economically produced in a manner suitable for therapeutic formulation and other uses. The present invention fulfills these and other needs. The hypervariable regions (also called Complementarity Determining Regions, abbreviated to "CDRs") of immunoglobulins were originally defined by Kabat et al., ("Sequences of Proteins of Immunological Interest" Kabat, E., et al., U.S. Department of Health and Human Services, (1983)) based on extent of sequence variability, to consist of residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain (V_L) and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain (V_H), using Kabat's standard numbering system for antibody amino acids. The CDRs are believed to contact the target antigen of an antibody and to be primarily responsible for binding. More recently Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901- 917 (1987)) have given an alternate definition of the hypervariable regions or CDRs as consisting of residues 26-32 (L1), 50-52 (L2), 91-96 (L3) in V_L and residues 26-32 (H1), 53- 55 (H2), 96-101 (H3) in V_H. The Chothia definition is based on the residues that constitute the loops in the 3-dimensional structures of antibodies. It is particularly important to note that for each of the six CDRs the Chothia CDR is actually a subset of (i.e. smaller than) the Kabat CDR, with the single exception of H1 (the first heavy chain CDR), where the Chothia CDR contains amino acids 26-30 that are not in the Kabat CDR.

Riechmann et al ("Reshaping human antibodies for therapy", Nature, Vol 332, pp 323-326, (March 1988)) describe work in which precisely the Kabat CDRs were transferred to a pre-determined human framework (NEW again for the heavy chain and REI for the light chain). However, they found that an antibody containing the humanized heavy chain lost most of its binding affinity and ability to lyse target cells. They therefore made a new humanized antibody containing the Kabat CDRs from the mouse antibody and two amino acid changes in Chothia CDR H1, but no other mouse amino acids.

Summary of the Invention

The invention provides the use of at least one amino acid substitution outside of complementarity determining regions (CDR's) as defined by Kabat et al ("Sequences of Proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)) together with Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)) in the production of a humanized immunoglobulin, wherein said at least one amino acid substitution is from the non-CDR variable region of a non-human donor immunoglobulin, and in which humanized immunoglobulin the variable region amino acid sequence other than the CDR's comprises at least 70 amino acid residues identical to an acceptor human immunoglobulin variable region amino acid sequence, and the CDR's are from the variable region of said non-human donor immunoglobulin.

In another aspect, the invention provides a method of producing a humanized immunoglobulin chain having a framework region from a human acceptor immunoglobulin and complementarity determining regions (CDR's) from a donor immunoglobulin capable of binding to an antigen, said method comprising the steps of substituting at least one non-CDR framework amino acid of the acceptor immunoglobulin with a corresponding amino acid from the donor immunoglobulin at a position in the immunoglobulins where:

(a) the amino acid in the human framework region of the acceptor immunoglobulin is rare for said position and the corresponding amino acid in the donor immunoglobulin is common for said position in human immunoglobulin sequences; or

(b) the amino acid is immediately adjacent to one of the CDR's; or

(c) the amino acid is predicted to have a side chain atom capable of interacting with the antigen or with the CDR's of the humanized immunoglobulin.

The present invention provides novel compositions useful, for example, in the treatment of T-cell mediated human disorders, the compositions containing human-like immunoglobulins specifically capable of blocking the binding of human IL-2 to its receptor and/or capable of binding to the p55 Tac protein on human IL-2 receptors. The immunoglobulins can have two pairs of light chain/heavy chain complexes, typically at least one pair having chains comprising mouse complementarity determining regions functionally joined to human framework region segments. For example, mouse complementarity determining regions, with or without additional naturally-associated mouse amino acid residues, can be used to produce human-like antibodies capable of binding to the human IL-2 receptor at affinity levels stronger than

resistance gene.

Figure 8. Schematic diagram of the plasmid pHuLTAC used to express the humanized anti-Tac light chain. Relevant restriction sites are shown, and coding regions of the light chain are displayed as boxes. The direction of transcription from the Ig promoter is shown by an arrow.

Figure 9. Fluorocytometry of HUT-102 and Jurkat cells stained with anti-Tac antibody or humanized anti-Tac antibody followed respectively by fluorescein-conjugated goat anti-mouse Ig antibody or goat anti-human Ig antibody, as labeled. In each panel, the dotted curve shows the results when the first antibody was omitted, and the solid curve the results when first and second (conjugated) antibodies were included as described.

Figure 10. (A) Fluorocytometry of HUT-102 cells stained with 0-40 ng of anti-Tac, as indicated, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin. (B) Flurocytometry of HUT-102 cells stained with the indicated antibody, then with biotinylated anti-Tac, and then with phycoerythrin-conjugated avidin.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with one embodiment of the present invention, human-like immunoglobulins specifically reactive with desired epitopes, such as those on the IL-2 receptor on human T-cells, are provided. These immunoglobulins, which have binding affinities of at least about 10^8 m^{-1}, and preferably 10^9 M^{-1} to 10^{10} M^{-1} or stronger, are capable of, e.g., blocking the binding of IL-2 to human IL-2 receptors. The human-like immunoglobulins will have a human-like framework and can have complimentary determining regions (CDR's) from an immunoglobulin, typically a mouse immunoglobulin, specifically reactive with an epitope on p55 Tac protein. The immunoglobulins of the present invention, which can be produced economically in large quantities, find use, for example, in the treatment of T-cell mediated disorders in human patients by a variety of techniques.

The basic antibody strutural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25kD) and one "heavy" chain (about 50-70kD). The NH_2-terminus of each chain begins a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The COOH terminus of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as either kappa or lambda. Heavy chains are classified (and subclassified) as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 12 more amino acids. (See, generally, Fundamental Immunology, Paul, W., Ed., Chapter 7, pgs. 131- 166, Raven Press, N.Y. (1984)).

The variable regions of each light/heavy chain pair form the antibody binding site. The chains all exhibit the same general structure of relatively conserved framework regions joined by three hypervariable regions, also called CDR's (see, "Sequences of Proteins of Immunological Interest," Kabat, E., et al., U.S. Department of Health and Human Services, (1983); and Cholthia and Lesk, J. Mol. Biol., 196:901-917 (1987)). The CDR's from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope.

As used herein, the term "immunoglobulin" refers to a protein consisting of one or more polypeptides substantially encoded by immunoglobulin genes. The recognized immunoglobulin genes include the kappa, lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as the myriad immunoglobulin variable region genes. The immunoglobulins may exist in a variety of forms besides antibodies; including, for example, Fv, Fab, and $F(ab)_2$, as well as in single chains (e.g., Huston, et al., Proc. Nat. Acad. Sci. U.S.A., 85:5879-5883 (1988) and Bird, et al., Science, 242:423-426 (1988)). (See, generally, Hood, et al., "Immunology", Benjamin, N.Y., 2nd ed. (1984), and Hunkapiller and Hood, Nature, 323:15-16 (1986)).

Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin gene segments belonging to different species. For example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments, such as γ_1 and γ_3. A typical therapeutic chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C or effector domain from a human antibody (e.g., A.T.C.C. Accession No. CRL 9688 secretes an anti-Tac chimeric antibody), although other mammalian species may be used.

As used herein, the term "framework region" refers to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved (i.e., other than the CDR's) among different immunoglobulins in a single species, as defined by Kabat, et al., op. cit. As used herein, a "human-like framework region" is a framework region that in each existing chain comprise at least about 70 or more amino acid residues, typically 75 to 85 or more residues, identical to those in a human immunoglobulin.

As used herein, the term "human-like immunoglobulin" refers to an immunoglobulin comprising a human-like framework and in which any constant region present is substantially homologous to a human immunoglobulin constant region, i.e., at least about-85-90%, preferably about 95% identical. Hence, all parts of a human-like immunoglobulin, except possibly the CDR's, are substantially homologous to corresponding parts of one or more native human immunoglobulin

bonding, Van der Waals forces, hydrophobic interactions, etc. At those amino acid positions, the donor amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will generally have a side chain atom within about 3 angstrom units of some site in the CDR's and must contain atoms that could interact with the CDR atoms according to established chemical forces, such as those listed above. Computer programs to create models of proteins such as-antibodies are generally available and well known to those skilled in the art (see, Loew et al., Int. J. Quant. Chem., Quant. Biol. Symp., 15:55-66 (1988); Bruccoleri et al., Nature, 335, 564-568 (1988); Chothia et al., Science, 233:755-758 (1986)). These do not form part of the invention. Indeed, because all antibodies have similar structures, the known antibody structures, which are available from the Brookhaven Protein Data Bank, can be used if necessary as rough models of other antibodies. Commercially available computer programs can be used to display these models on a computer monitor, to calculate the distance between atoms, and to estimate the likelihood of different amino acids interacting (see, Ferrin et al., J. Mol. Graphics, 6:13-27 (1988)).

Humanized or human-like antibodies generally have at least three potential advantages over mouse or in some cases chimeric antibodies for use in human therapy:

1) Because the effector portion is human, it may interact better with the other parts of the human immune system (e.g., destroy the target cells more efficiently by complement- dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC)).

2) The human immune system should not recognize the framework or constant region of the humanized antibody as foreign, and therefore the antibody response against such an injected antibody should be less than against a totally foreign mouse antibody or a partially foreign chimeric antibody.

3) Injected mouse antibodies have been reported to have a half-life in the human circulation much shorter than the half-life of normal antibodies (D. Shaw et al., J. Immunol., 138:4534-4538 (1987)). Injected humanized antibodies will presumably have a half-life more similar to naturally occurring human antibodies, allowing smaller and less frequent doses to be given.

The present invention is specifically directed to improved humanized immmunoglobulins (e.g., capable of binding the human IL-2 receptor) with respect to those described in EPA publication no. 0239400. That application, the disclosure of which is excluded from coverage herein, describes, for certain immunoglobulins substituting CDR's regions in the light or heavy chain variable domains of an acceptor antibody with analogous parts of CDR's (typically solvent accessible) from an antibody of different specificity. Also, that application discusses, for certain immunoglobulins, the possibility of only transferring residues that are (solvent) accessible from the antigen binding site, which residues apparently may include certain framework regions (specifically, residues known to be involved in antigen binding as described in Amit et al., Science 233: 747-753 (1986) or perhaps residues essential for inter-chain interactions - but for the selection of which insufficient guidance is provided in that application). Thus, for example, a preferred embodiment of the present invention entails substituting entire CDR's and framework amino acids immediately adjacent one (or preferably each) of the CDR's. In general, any framework residue that also makes contact with the CDR's to, e.g., maintain their conformation (and usually their antigen binding specificity) are specifically included within preferred embodiments of the present invention as described in detail, supra.

In one aspect, the present invention is directed to recombinant DNA segments encoding the heavy and/or light chain CDR's (typically with other amino acid residues as described above) from an immunoglobulin capable of binding to a desired epitope, such as on the human IL-2 receptor (e.g., the anti-Tac monoclonal antibody). The DNA segments encoding these regions will typically be joined to DNA segments encoding appropriate human-like framework regions. For example, the preferred DNA sequences, which on expression code for the polypeptide chains comprising the anti-Tac heavy and light chain hypervariable regions (with human-like framework regions), are shown in Figures 3 and 4, respectively. Due to codon degeneracy and non-critical amino-acid substitutions, other DNA sequences can be readily substituted for those sequences, as detailed below.

The DNA segments will typically further include an expression control DNA sequence operably linked to the human-like antibody coding sequences, including naturally-associated or heterologous promoter regions. Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow.

Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B-cells (see, Kabat op. cit. and WP87/02671). For example, the human kappa immunoglobulin constant and J region genes and sequences are described in Heiter et al.,. Cell 22:197-207 (1980) and the nucleotide sequence of a human immunoglobulin $C_{\gamma 1}$ gene is described in Ellison et al., Nucl. Acid. Res. 10:4071 (1982). The CDR's for producing the immunoglobulins of the present invention will be similarly derived from

homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the polypeptides may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures, immunofluorescent stainings, and the like. (See, generally, Immunological Methods, Vols. I and II, Lefkovits and Pernis, eds., Academic Press, New York, N.Y. (1979 and 1981)).

The IL-2 receptor specific antibodies exemplified in the present invention will typically, find use individually in treating a T-cell mediated disease state. Generally, where the cell linked to a disease has been identified as IL-2 receptor bearing, then the human-like antibodies capable of blocking the binding of IL-2 to the human IL-2 receptor are suitable. For example, typical disease states suitable for treatment include graft versus host disease and transplant rejection in patients undergoing an organ transplant, such as heart, lungs, kidneys, liver, etc. Other diseases include autoimmune diseases, such as Type I diabetes, multiple sclerosis, rheumatoid arthritis, systemic lupus erythematosus, and myasthenia gravis.

The human-like antibodies of the present invention may also be used in combination with other antibodies, particularly human monoclonal antibodies reactive with other markers on cells responsible for the disease. For example, suitable T-cell markers can include those grouped into the so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Bernard, et al., Eds., Springer-Verlag, N.Y. (1984).

The antibodies can also be used as separately administered compositions given in conjunction with chemotherapeutic or immunosuppressive agents. Typically, the agents will include cyclosporin A or a purine analog (e.g., methotrexate, 6-mercaptopurine, or the like), but numerous additional agents (e.g., cyclophosphamide, prednisone, etc.) well-known to those skilled in the art may also be utilized.

A preferred pharmaceutical composition of the present invention comprises the use of the subject antibodies in immunotoxins. Immunotoxins are characterized by two components and are particularly useful for killing selected cells in vitro or in vivo. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle," provides a means for delivering the toxic agent to a particular cell type, such is cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known with the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982).

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatin; and cytotoxic proteins such as ribosomal inhibiting proteins like pokeweed antiviral protein, Pseudomonas exotoxin A, ricin, diphtheria toxin, ricin A chain, etc., or an agent active at the cell surface, such as the phospholipase enzymes (e.g., phospholipase, (See, generally, "Chimeric Toxins," Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985)).

The delivery component of the immunotoxin will include the human-like immunoglobulins of the present invention. Intact immunoglobulins or their binding fragments, such as Fab, are preferably used. Typically, the antibodies in the immunotoxins will be of the human IgM or IgG isotype, but other mammalian constant regions may be utilized as desired.

The human-like antibodies and pharmaceutical compositions thereof of this invention are particularly useful for parenteral administration, i.e., subcutaneously, intramuscularly or intravenously. The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, e.g., water, buffered water, 0.4% saline, 0.3% glycine and the like. These solutions are sterile and generally free of particulate matter. These compositions may be sterilized by conventional, well known sterilization techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjusting agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, i.e., from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made upt to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringer's solution, and 150 mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pennsylvania (1980).

The antibodies of this invention can be lyophilized for storage and reconstituted in suitable carrier prior to use. This technique has been shown to be effective with conventional immune globulins and art-known lyophilization and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution

(2) The Eu amino acid was unusual for human heavy chains at that position, whereas the anti-Tac amino acid was typical for human heavy chains at that position (amino acids 27, 93, 95, 98, 107-109, 111);
(3) The position was immediately adjacent to a CDR in the amino acid sequence of the anti-Tac heavy chain (amino acids 30 and 67).
(4) 3-dimensional modeling of the anti-Tac antibody suggested that the amino acid was physically close to the antigen binding region (amino acids 48 and 68).

Some amino acids fell in more than one of these categories but are only listed in one.

To select the sequence of the humanized light chain, the anti-Tac light chain sequence was aligned with the sequence of the Eu light chain (Figure 2). The Eu amino acid was selected at each position, unless the position again fell into one of the categories (1) - (4), (with light chain replacing heavy chain in the category definitions):

(1) CDRs (amino acids 24-34, 50-56, 89-97).
(2) Anti-Tac amino acid more typical than Eu (amino acids 48 and 63).
(3) Adjacent to CDRs (no amino acids; Eu and anti-Tac were already the same at all these positions).
(4) Possible 3-dimensional proximity to binding region (amino acid 60).

The actual nucleotide sequence of the heavy (Figure 3) and light chain (Figure 4) genes were selected as follows:

(1) the nucleotide sequences code for the amino acid sequences chosen as described above.
(2) 5' of these coding sequences, the nucleotide sequences code for a leader (signal) sequence, namely the leader of the light chain of the antibody MOPC 63 and the leader of the heavy chain of the antibody PCH 108A (Kabat et al., op. cit.). These leader sequences were chosen as typical of antibodies.
(3) 3' of the coding sequences, the nucleotide sequences are the sequences that follow the mouse light chain J5 segment and the mouse heavy chain J2 segment, which are part of the anti-Tac sequences. These sequences are included because they contain splice donor signals.
(4) At each end of the sequence is an Xba I site to allow cutting at the Xba I sites and cloning into the Xba I site of a vector.

Construction of humanized light and heavy chain genes

To synthesize the heavy chain, four oligonucleotides HES12, HES13, HES14, HES15 (Figure 5A) were synthesized using an Applied Biosystems 380B DNA synthesizer. Two of the oligonucleotides are part of each strand of the heavy chain, and each oligonucleotide overlaps the next one by about 20 nucleotides to allow annealing (Figure 5B). Together, the oligonucleotides cover the entire humanized heavy chain (Figure 3) with a few extra nucleotides at each end to allow cutting at the Xba I sites. The oligonucleotides were purified from polyacrylamide gels.

Each oligonucleotide was phosphorylated using ATP and T4 polynucleotide kinase by standard procedures (see, Maniatis, op. cit.). To anneal the phosphorylated oligonucleotides, they were suspended together in 40 µl of TA (33 mM Tris acetate, pH 7.9, 66 mM potassium acetate, 10 mM magnesium acetate) at a concentration of about 3.75 uM each, heated to 95 deg for 4 min. and cooled slowly to 4 °C. To synthesize the complete gene from the oligonucleotides by synthesizing the opposite strand of each oligonucleotide (Figure 5B), the following components were added in a final volume of 100µl:

10 µl	annealed oligonucleotides
0.16 mM each	deoxyribonucleotide
0.5 mM	ATP
0.5 mM	DTT
100 µg/ml	BSA
3.5 µg/ml	T4 g43 protein (DNA polymerase)
25 µg/ml	T4 g44/62 protein (polymerase accessory protein)
25 µg/ml	45 protein (polymerase accessory protein)

The mixture was incubated at 37 °C for 30 min. Then 10 U of T4 DNA ligase was added and incubation at 37 °C resumed for 30 min. The polymerase and ligase were inactivated by incubation of the reaction at 70 °C for 15 min. To digest the gene with Xba I, to the reaction was added 50 µl of 2x TA containing BSA at 200 µg/ml and DTT at 1 mM, 43 µl of water, and 50 U of Xba I in 5 µl. The reaction was incubated for 3 hr at 37 °C, and run on a gel. The 431 bp Xba I fragment was purified from a gel and cloned into the Xba I site of the plasmid pUC19 by standard methods. Four plasmids

Biological properties of the humanized antibody

For optimal use in treatment of human disease, the humanized antibody should be able to destroy T-cells in the body that express the IL-2 receptor. One mechanism by which antibodies may destroy target cells is antibody-dependent cell-mediated cytotoxicity, abbreviated ADCC (Fundamental Immunology, Paul, W., Ed., Raven Press, New York (1984), at pg. 681), in which the antibody forms a bridge between the target cell and an effector cell such as a macrophage that can lyse the target. To determine whether the humanized antibody and the original mouse anti-Tac antibody can mediate ADCC, a chromium release assay was performed by standard methods. Specifically, human leukemia HUT-102 cells, which express the IL-2 receptor, were incubated with ^{51}Cr to allow them to absorb this radionuclide. The HUT-102 cells were then incubated with an excess of either anti-Tac or humanized anti-Tac antibody. The HUT-102 cells were next incubated for 4 hrs with either a 30:1 or 100:1 ratio of effector cells, which were normal purified human peripheral blood mononuclear cells that had been activated by incubation for about 20 hrs with human recombinant IL-2. Release of ^{51}Cr, which indicated lysis of the target HUT-102 cells, was measured and the background subtracted (Table 1). The results show that at either ratio of effector cells, anti-Tac did not lyse a significant number of the target cells (less than 5%), while the humanized antibody did (more than 20%). Hence, the humanized antibody is likely to be more efficacious than the original mouse antibody in treating T-cell leukemia or other T-cell mediated diseases.

TABLE 1

Percent ^{51}Cr release after ADCC		
Effector: Target ratio		
	30:1	100:1
Antibody		
Anti-Tac	4%	< 1%
Humanized anti-Tac	24%	23%

From the foregoing, it will be appreciated that the human-like immunoglobulins of the present invention offer numerous advantages of other antibodies. For example, in comparison to anti-Tac mouse monoclonal antibodies, the present human-like IL-2 receptor immunoglobulins can be more economically produced and contain substantially less foreign amino acid sequences. This reduced likelihood of antigenicity after injection into a human patient represents a significant therapeutic improvement for immunoglobulins designed in accordance with the above criteria.

Claims

1. The use of at least one amino acid substitution outside of complementarity determining regions (CDR's) as defined by Kabat et al ("Sequences of Proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)) together with Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)) in the production of a humanized immunoglobulin, wherein said amino acid substitution is from the non-CDR variable region of a non-human donor immunoglobulin, and in which humanized immunoglobulin the variable region amino acid sequence other than the CDR's comprises at least 70 amino acid residues identical to an acceptor human immunoglobulin variable region amino acid sequence, and the CDR's are from the variable region of said non-human donor immunoglobulin.

2. A use according to claim 1, wherein said humanized immunoglobulin is specifically reactive with p55 Tac protein, is capable of inhibiting binding of human interleukin-2 (IL-2) to a human IL-2 receptor, or is capable of binding to a human IL-2 receptor.

3. A use according to claim 2, wherein said humanized immunoglobulin exhibits a binding affinity to a human IL- 2 receptor of about 10^8 M^{-1} or stronger.

4. A use according to claim 2 or claim 3, wherein the mature light and heavy variable region protein sequences of said humanized immunoglobulin are homologous to the mature protein sequences in Figures 3 and 4.

5. A use according to any one of claims 1 to 4, wherein said humanized immunoglobulin is an IgG_1 immunoglobulin isotype.

den Regionen (CDR's), wie bei Kabat et al. ("Sequences of Proteins of Immunological Interest", Kabat, E., et al., US Department of Health and Human Services, (1983)) zusammen mit Chothia et al (Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987)) definiert, für die Herstellung von einem humanisierten Immunglobulin, in dem die Aminosäure-Substitution von der nicht-CDR-variablen Region von einem nicht menschlichen Donor-Immunglobulin ist, und in dem die Aminosäuresequenz der variablen Region des humanisierten Immunglobulins anders als die CDR's mindestens 70 Aminosäurereste identisch zu einer Aminosäuresequenz der variablen Region von einem menschlichen Akzeptor-Immunglobulin umfassen, und in dem die CDR's von der variablen Region von dem nicht menschlichen Donor-Immunglobulin sind.

2. Verwendung nach Anspruch 1, wobei das humanisierte Immunglobulin spezifisch reaktiv für p55 TAC-Protein ist, fähig ist, die Bindung von menschlichem Interleukin-2 (IL-2) zu einem menschlichem IL-2-Rezeptor zu verhindern, oder zur Bindung zu einem menschlichen IL-2-Rezeptor fähig ist.

3. Verwendung nach Anspruch 2, wobei das humanisierte Immunglobulin eine Bindungsaffinität zu einem menschlichen IL-2-Rezeptor von etwa 10^8 M^{-1} oder mehr zeigt.

4. Verwendung nach Anspruch 2 oder 3, wobei die Proteinsequenzen der leichten und schweren variablen Region von dem humanisierten Immunglobulin homolog zu der Sequenz des reifen Proteins in Figur 3 und 4 sind.

5. Verwendung nach einem der Ansprüche 1 - 4, wobei das humanisierte Immunglobulin ein IgG_1 Immunglobulin-Isotyp ist.

6. Verwendung nach einem der Ansprüche 1 - 5, wobei die Substitution unmittelbar an eine CDR angrenzt.

7. Ein Verfahren zur Herstellung einer humanisierten Immunglobulinkette mit einer Rahmenregion von einem menschlichen Akzeptor-Immunglobulin und mit die Komplementarität bestimmenden Regionen (CDR's) von einem Donor-Immunglobulin, die fähig sind, an ein Antigen zu binden, wobei das Verfahren die Substitution von mindestens einer nicht-CDR-Rahmen-Aminosäure des Akzeptor-Immunglobulins durch eine korrespondierende Aminosäure von dem Donor-Immunglobulin an einer Position in den Immunglobulinen umfaßt, wobei:

   (a) die Aminosäure in der menschlichen Rahmenregion des Akzeptor-Immunglobulins selten für diese Position ist und die korrespondierende Aminosäure in dem Donor-Immunglobulin häufig für diese Position in menschlichen Immunglobulinsequenzen ist, oder

   (b) die Aminosäure unmittelbar benachbart zu einer der CDR's ist oder

   (c) die Aminosäure ein Seitenkettenatom hat, das fähig ist, mit dem Antigen oder mit den CDR's des humanisierten Immunglobulins zu interagieren.

8. Ein Verfahren nach Anspruch 7, in dem mindestens drei der nicht CDR-Rahmen-Aminosäuren substituiert sind durch Aminosäuren des Donor-Immunglobulins, ausgewählt nach den Kriterien (a), (b) oder (c).

9. Ein Verfahren nach Anspruch 8, wobei mindestens eine der durch den Donor substituierten Aminosäuren unmittelbar an eine CDR angrenzt.

10. Ein Verfahren nach einem der Ansprüche 7 - 9, wobei die Proteinsequenzen der reifen leichten und schweren variablen Region dieses humanisierten Immunglobulins homolog sind zu den Sequenzen des reifen Proteins in Figur 3 und 4.

11. Eine humanisierte Immunglobulinkette, erhältlich bei einer Verwendung nach einem der Ansprüche 1 - 6.

12. Eine humanisierte Immunglobulinkette, erhältlich bei einem Verfahren nach einem der Ansprüche 7 - 10.

13. Ein humanisiertes Immunglobulin, in dem die schweren und leichten Ketten Ketten nach Anspruch 11 oder 12 sind.

14. Ein Polynucleotid umfassend eine erste Sequenz kodierend für eine nicht- CDR-Rahmenregion eines menschenähnlichen Immunglobulins und eine zweite Sequenz kodierend für eine oder mehrere CDR's, wobei das Polynucleotid eine Immunoglobulinkette nach Anspruch 11 oder 12 kodiert.

(c) l'amino acide est supposé avoir un atome sur une chaîne latérale capable d'interagir avec l'antigène ou avec les CDR de l'immunoglobuline humanisée.

8. Méthode selon la revendication 7 où il y a au moins trois des dix amino acides de la région charpente non-CDR qui sont substitués par des amino acides provenant de l'immunoglobuline donneur choisie en fonction des critères (a), (b) ou (c).

9. Méthode selon la revendication 8 où au moins l'un des amino acides substitués provenant du donneur est immédiatement adjacent à une CDR.

10. Méthode selon l'une quelconque des revendications 7 à 9 où les séquences protéiques matures des régions variables légère et lourde de ladite immunoglobuline humanisée sont homologues aux séquences protéiques matures des figures 3 et 4.

11. Chaîne d'immunoglobuline humanisée qui peut être obtenue par une utilisation selon l'une quelconque des revendications 1 à 6.

12. Chaîne d'immunoglobuline humanisée qui peut être obtenue par une méthode selon l'une quelconque des revendications 7 à 10.

13. Immunoglobuline humanisée dans laquelle les chaînes lourdes et légères sont des chaînes selon la revendications 11 ou 12.

14. Polynucléotide comprenant une première séquence codant pour une région charpente non-CDR d'une immunoglobuline semblable à une région humaine ("human-like") et une seconde séquence codant pour une ou plusieurs CDR où lors de son expression ledit polynucléotide code une chaîne d'immunoglobuline selon la revendication 11 ou la revendication 12.

15. Polynucléotides selon la revendication 14 qui lors de leur expression codent les chaînes constituant une immunoglobuline selon la revendication 13.

16. Lignée cellulaire transfectée avec un polynucléotide ou des polynucléotides selon la revendication 14 ou 15.

17. Procédé pour la préparation d'une immunoglobuline humanisée tel que défini dans la revendication 13 comprenant la culture d'une lignée cellulaire telle que définie dans la revendication 16 et l'isolement de l'immunoglobuline humanisée du milieu de culture cellulaire.

18. Utilisation d'une immunoglobuline selon la revendication 13 ou d'un fragment de liaison de celle-ci pour la fabrication d'un médicament.

19. Utilisation selon la revendication 18 où le médicament est adapté au traitement des désordres médiés par des cellules T chez un patient humain.

20. Préparation pharmaceutique qui contient une immunoglobuline humanisée selon la revendication 13 formulée sous une forme pharmaceutiquement acceptable.

21. Préparation pharmaceutique selon la revendication 20 et pour le traitement des désordres médiés par des cellules T.

```
        10         20         30         40         50         60
TCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTGCACT
      M  G  W  S  W  I  F  L  F  L  L  S  G  T  A  G  V  H

        70         80         90        100        110        120
CTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTGAAGG
S  Q  V  Q  L  V  Q  S  G  A  E  V  K  K  P  G  S  S  V  K

       130        140        150        160        170        180
TCTCCTGCAAGGCTTCTGGCTACACCTTTACTAGCTACAGGATGCACTGGGTAAGGCAGG
V  S  C  K  A  S  G  Y  T  F  T  S  Y  R  M  H  W  V  R  Q

       190        200        210        220        230        240
CCCCTGGACAGGGTCTGGAATGGATTGGATATATTAATCCGTCGACTGGGTATACTGAAT
A  P  G  Q  G  L  E  W  I  G  Y  I  N  P  S  T  G  Y  T  E

       250        260        270        280        290        300
ACAATCAGAAGTTCAAGGACAAGGCAACAATTACTGCAGACGAATCCACCAATACAGCCT
Y  N  Q  K  F  K  D  K  A  T  I  T  A  D  E  S  T  N  T  A

       310        320        330        340        350        360
ACATGGAACTGAGCAGCCTGAGATCTGAGGACACCGCAGTCTATTACTGTGCAAGAGGGG
Y  M  E  L  S  S  L  R  S  E  D  T  A  V  Y  Y  C  A  R  G

       370        380        390        400        410        420
GGGGGGTCTTTGACTACTGGGGCCAAGGAACCCTGGTCACAGTCTCCTCAGGTGAGTCCT
G  G  V  F  D  Y  W  G  Q  G  T  L  V  T  V  S  S

       430
TAAAACCTCTAGA
```

FIG._3.

A

HES12 AGCTTCTAGATGGGATGGAGCTGGATCTTTCTCTTCCTCCTGTCAGGTACCGCGGGCGTG
CACTCTCAGGTCCAGCTTGTCCAGTCTGGGGCTGAAGTCAAGAAACCTGGCTCGAGCGTC
AAGGTC

HES13 CCCAGTCGACGGATTAATATATCCAATCCATTCCAGACCCTGTCCAGGGGCCTGCCTTAC
CCAGTGCATCCTGTAGCTAGTAAAGGTGTAGCCAGAAGCCTTGCAGGAGACCTTCACGCT
CGAGCCAGG

HES14 TATATTAATCCGTCGACTGGGTATACTGAATACAATCAGAAGTTCAAGGACAAGGCAACA
ATTACTGCAGACGAATCCACCAATACAGCCTACATGGAACTGAGCAGCCTGAGATCTGAG
GACA

HES15 ATATCGTCTAGAGGTTTTAAGGACTCACCTGAGGAGACTGTGACCAGGGTTCCTTGGCCC
CAGTAGTCAAAGACCCCCCCCCCTCTTGCACAGTAATAGACTGCGGTGTCCTCAGATCTC
AGGCTGCT

B

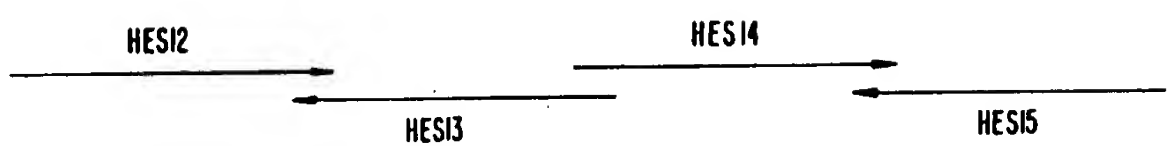

FIG._5.

EP 0 451 216 B1

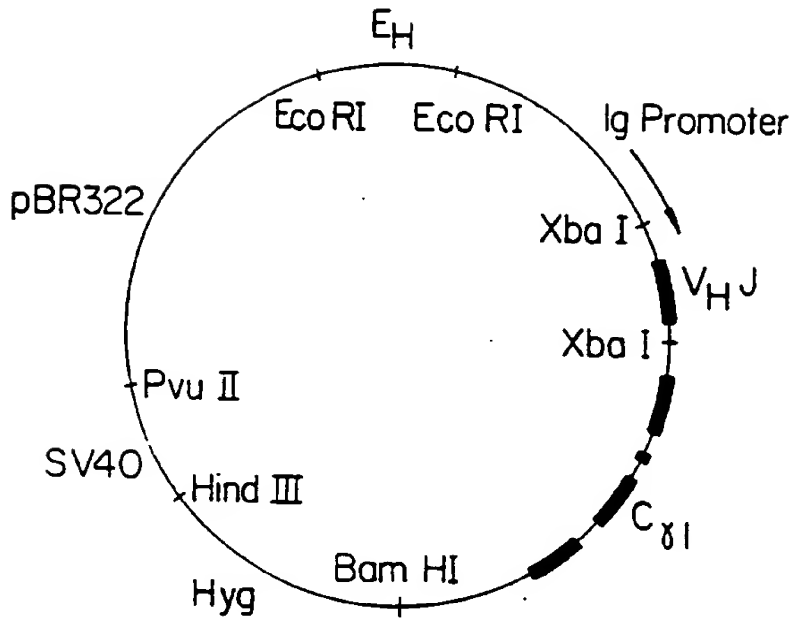
E_H
Eco RI
Eco RI
Ig Promoter
pBR322
Xba I
$V_H J$
Xba I
Pvu II
SV40
Hind III
$C_{\gamma 1}$
Bam HI
Hyg

FIG._7.

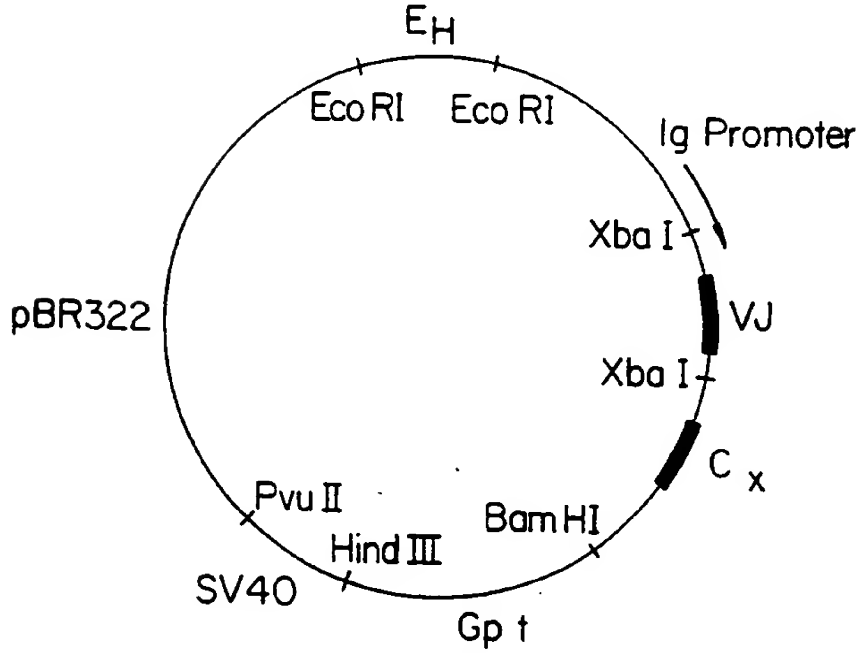
E_H
Eco RI
Eco RI
Ig Promoter
Xba I
pBR322
VJ
Xba I
C_x
Pvu II
Bam HI
Hind III
SV40
Gp t

FIG._8.

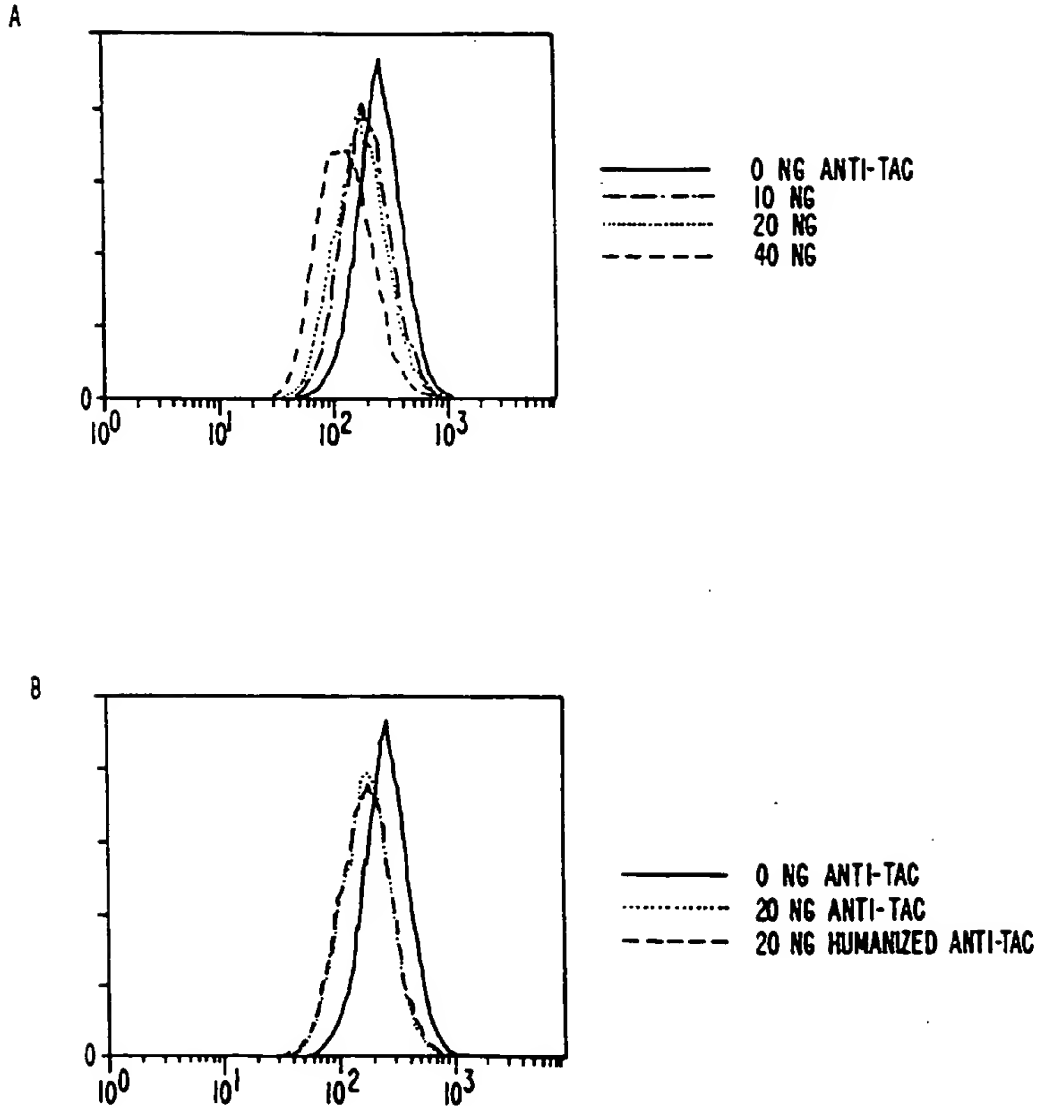
A
0
10^0
10^1
10^2
10^3
0 NG ANTI-TAC
10 NG
20 NG
40 NG
B
0
10^0
10^1
10^2
10^3
0 NG ANTI-TAC
20 NG ANTI-TAC
20 NG HUMANIZED ANTI-TAC

FIG._10.

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